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Role of silver nanoparticles in countering negative effects generated by PEG induced drought stress in Pearl millet (*Pennisetum glaucum* L.) R.Br

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Abstract

Millets are becoming an important crop for food security aiding in the fight against poverty and malnutrition in arid and semi-arid regions. It has become a staple grain for millions of people in West Africa and India. Drought stress included as one of the most important environmental constrains to limits the growth and yield of the crop plants. Seed germination initiate from the imbibition of water which activates the physiological processes that's where plant's life start. A study was conducted to investigate the effect of silver nanoparticles (Ag NPs) to ameliorating the negative effects caused by drought stress on germination and growth of pearl millet seeds. Ag NPs can improve seed germination and contribute to plant tolerance of drought stress toxicity. During current experiment, the efficacy of Ag NPs was assessed for reducing drought toxicity in *Pearl millet*. In our experiment, seed germination, shoot length and root length, germination index, and vigor index were calculated with/without application of Ag NPs (50 ppm) in the presence/absence of polyethylene glycol (20%) in *Pearl millet*. Application of Ag NPs, in the presence of PEG, enhanced seed germination, seedling growth, germination index, and vigor index significantly compared to the seeds treated with PEG. These results depicted that application of Ag NPs, could be a useful approach to assist PEG confiscation and stress tolerance against drought in *Pearl millet* in drought prone areas.

Key words : Nanotechnology, Silver nanoparticles, Drought stress, Pearl millet, Seed germination, PEG.

In the developing world, millets are becoming one of the major crops, predominantly where they are utilised for food for human and fodder for animals[18]. They can sustain adversity in agro climatic conditions hence are considered as important crop for food security. These crops are not only potential in increasing the genetic diversity in the food basket but also safeguard food and national security. Millets offer many health benefits and provides help in managing chronic disorders like diabetes mellitus, obesity, hyperlipidemia etc, along with increasing nutritional quality of the food intake on daily basis[8]. Thus, resulting in a rise in cultivation and production of millets. According to a report by Food and Agricultural Organization (FAO) in 2010 India top the ranking by producing 3, 34,500 tonnes of total 7, 62,712 tonnes of millets produced worldwide[13]. Millets are small-seeded with different varieties such as pearl millet (*Pennisetum glaucum*), kodo millet (*Paspalum setaceum*), finger millet (*Eleusine coracana*), barnyard millet (*Echinochloa utilis*). proso millet (*Panicum miliaceum*), little millet (*Panicum sumatrense*) and foxtail millet (*Setaria italic*)[2]. They are known as coarse cereals beside sorghum (*Sorghum bicolor*), maize (*Zea mays*), barley (*Hordeum vulgare*) and oats (*Avena sativa*)[13]. Among these millets pearl millet *Pennisetum glaucum* L. is the most widely grown minor cereal crop in the world[11]. It is a nutritious and staple cereal of the arid and semi-arid areas of the world. It stands sixth in world and fourth in India most important cereals. It is grown in bout 27 million hectares worldwide.

Drought is one of the common among other abiotic stresses which poses a major constraint to the production of pearl millet across the globe. In India 67% of the net sown areas production is limited due to water deficit[12]. Drought not only affects seed germination, seedling growth, plant growth but also the overall plant yield. In laboratory experiments PEG is used for induction of drought stress. To screen the drought tolerant germplasm it has been used in many experiments as a stress inducer PEG is a non-penetrable and non-toxic osmotic substance which is used to lower the water potential and it has been used to simulate drought stress[11]. Now a days NPs are been used widely in agricultural systems for alleviating the impact of drought on plants by increasing anti-oxidant enzyme activity, affecting physiological properties and improving the phytohormone levels[3].

The use of NPs in recent years increased significantly. NPs showed both positive as well as negative impacts on morphological, physiological, and biochemical parameters of the plants. Performance of NPs in plants is depends upon the concentration, and types of the NPs, it also influenced by the plant species. Different types of NPs are used in agriculture but most common NPs used are silver (Ag) and zinc (Zn)[3].

In the present experiment, we aimed to assess the efficacy of exogenous Ag-NPs in *Pearl millet* under PEG stress. We mainly pay attention on how Ag-NPs alter % germination, shoot and root length, germination index and vigor index.

The experiment was conducted in the Lab of Botany Section, School of Sciences, Maulana Azad National Urdu University, Hyderabad. The seeds used in the experiment were procured from Indian Institute of Millet Research (IIMR), Hyderabad. PEG was obtained from the Ashwini chemical labs Secunderabad. The molecular weight of PEG used in this experiment is 6000. Ag-NPs were obtained from Nano Research Lab, Jamshedpur, Jharkhand. Healthy and uniform seeds of pearl millet were surface sterilized with 1% sodium hypochlorite for 5 min, and washed with distilled water and surface dried. Twenty seeds for each cultivar in each treatment were allowed to germinate on filter paper in Petri dishes. Treatments were control, 20% of PEG (MW 6000), 50 ppm of Ag-NPs, and 20% of PEG + 50 ppm of Ag-NPs. A quantity of 4 ml of appropriate solution was applied to each Petri dish. The Petri plates were arranged in completely randomized design (CRD) with three replicates of each treatment. The petri dishes were then kept in incubator. The temperature was maintained at 28 ± 2°C. The plates were checked periodically and respective solutions were applied to compensate any evaporation. Seeds were considered germinated when the radicle length was at least 2 mm visible. The parameters recorded are germination percentage, germination index, root length, shoot length and vigor index. The calculations were done based on the following formulas:

$$\text{Germination\%}=\frac{no\ of\ seeds\ germinated}{no\ of\ seeds\ tested}\times 100$$

Germination index is calculated by the formula given by Tao and Zheng[17]

$$germination\ index=\frac{germination\ \%}{no\ of\ days\ of\ germination}$$

Vigor index is calculated by the formula given by Zhu and Hong[20]

$$vigor\ index = germination\% \times mean\ of\ seedling\ length(root+shoot)$$

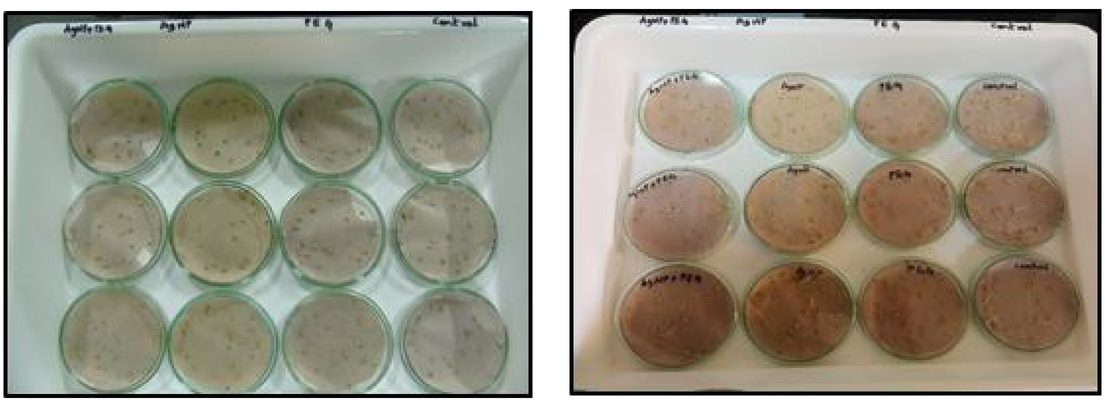

Figure 1. Experimental setup before and after germination.

It is observed from the figure 2 that germination % was varied among all the treatments with the highest recorded in the control *i.e.*, 98.3% and almost similar in the seeds which were treated only with AgNP. There was a significant decrease in germination % in the seeds treated with PEG which showed an average of 38.3%. The seeds which were treated with AgNP +PEG showed a significant increase by producing a germination % of 95.6%.

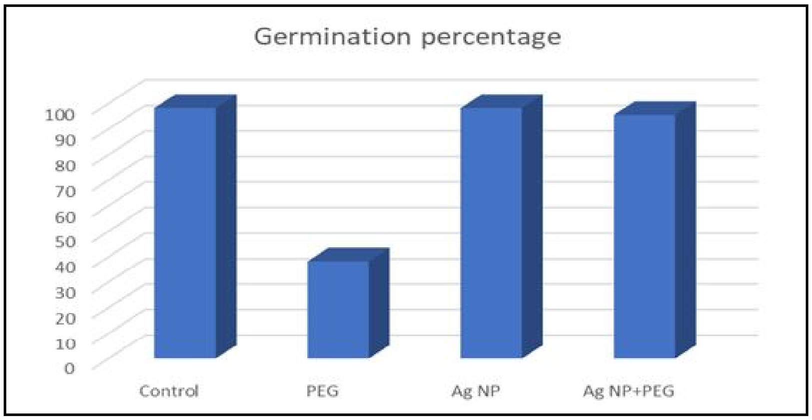

Figure 2. Graph representing germination percentage of Pearl millets seeds under different treatments.

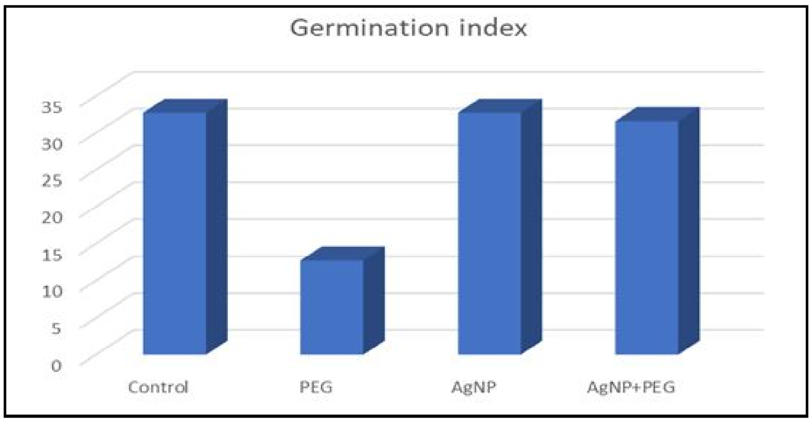

Figure 3. Graph representing germination index of Pearl millet under different treatments.

Germination index :

The data presented in figure 3 showed a similar trend as of germination % with a highest index in control followed by the seeds which were treated only with AgNP, a significant decrease in the germination index was seen in seeds treated with PEG but the seeds treated with AgNP +PEG again showed a positive effect by increasing the germination index.

Shoot length :

In figure 4 data of shoot lengths is represented. It demonstrates that the shoot length considerably decreased with an average value of 2.74cm in the treatments consists only of PEG solution. Control showed the highest shoot length with an average value of 7.24. when only AgNP solution was applied the average value of 5.23 was obtained. When AgNP was provided to the treatment with PEG

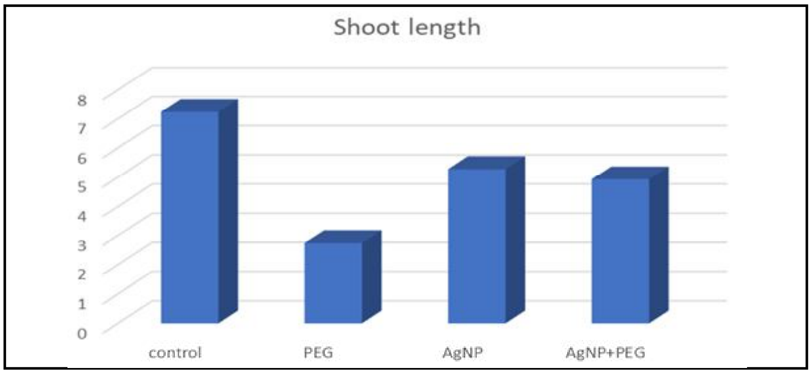

Figure 4. Graph representing shoot lengths of Pearl millet under different treatments

solution the shoot length considerably increases to an average value of 4.93 which was greater than that of seeds treated with only PEG solution showing countering effect of AgNP on PEG to increase the shoot length.

Root length :

Figure 5 shows the data collected on root length. The least root length was recorded in the seeds treated with only PEG solution with an average value of 4.76. The seeds treated with both AgNP and PEG solution showed the highest value with an average of 8.6 which was slightly above the control having 8.19. Seeds treated with only AgNP showed a normal response ranging approximately near the control values.

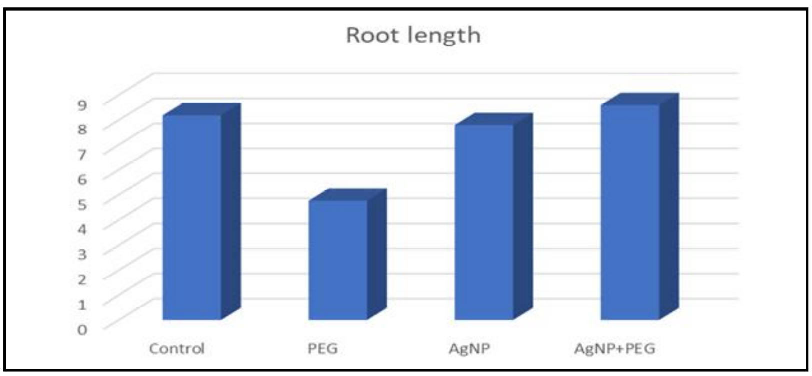

Figure 5. Graph representing the data on Root lengths of Pearl millet seeds under different treatments.

Vigor index :

The vigor index also showed a significant variation with the highest value in control and similar values in seeds treated with AgNP solution and the least shown in seeds treated with only PEG as shown in the fig 6. The seeds treated with AgNP + PEG solution showed a increasing trend approximately nearing the values of control illustrating the positive effects of AgNP.

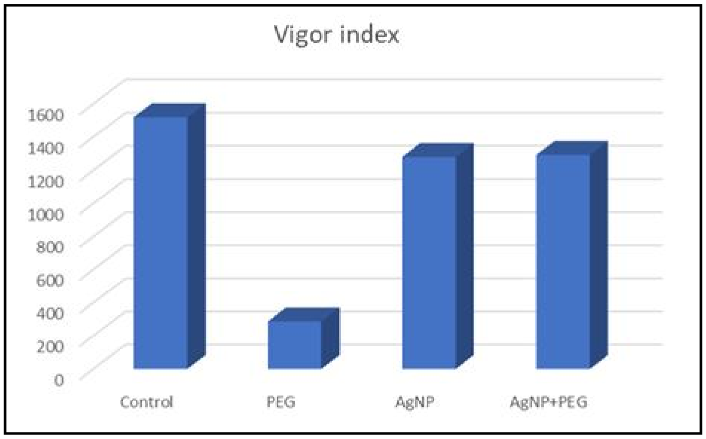

Figure 6. graph representing vigor index of Pearl millet seeds under different treatments.

Nanotechnology is an enthralling and swiftly developing division of research that has led to various innovations. In particular, nanotechnology can help provide effective solutions to agriculture-related problems and achieve a sustainable and secure future for agriculture[3]. Among various types of nanomaterials, silver nanoparticles (Ag NPs) are the most commonly applied nanomaterial.[19] PEG used in our study is an inductee of osmotic stress and the results we obtained are in conformity with several studies which showed that in vitro screening of plant with peg is a dependable factor to study plant reaction for water scarcity[1] and to select best variety of plant. Similar results were shown in study conducted by Daouda *et al.,*[14] and R. C. Meena *et al.,*[12] in-pearl millet and Naveena Sharon. R *et al.,*[16] in-finger millet. A similar trend was seen in different millets by Horalchova *et al.,*[7] and Senthil *et al.,*[15] also reported in maize by Zahra[10]. In our study AgNP showed a positive effect in countering the negative impacts incurred by the PEG induced drought, similar results in the germination parameters were demonstrated in an experiment by khan *et al.,*[9] in pearl millet. Significant increase by the application of AgNP was seen in lentils by Hojjat[6] and in Phaseolus and maize by Hediat[5]. The significant difference can be due to biochemical changes within the seeds which are required to start the germination process such as breaking of dormancy, hydrolysis or metabolization of inhibitors, imbibition and enzyme activation.[4] This can be used as base and can be applied on a large-scale basis to avoid any crop loss due to droughts and can be used as a regular practice in drought prone areas.

It is concluded from the present investigation that PEG significantly decreased the germination %, germination index, root length, shoot length and vigor index of Pearl millet. However, exogenous application of Ag NPs reduced the toxicity caused by PEG and increased the aforesaid parameters.

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